REMARKS

Claim 1 has been amended for greater clarity. The claim amendments are supported by the specification (e.g., page 12, lines 7-14; and Figure 1). No new matter has been introduced. The amendments are made solely to expedite prosecution of the application, and Applicants reserve the right to prosecute claims of similar or differing scope in subsequent applications.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

Claim Rejections under 35 U.S.C. § 102(b)

Claims 1-3 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Hotz et al. (Mol. Biochem. Parasitology, 75:1-14, 1995). Applicants respectfully traverse these rejections.

Independent claim 1 as amended is directed to a method for removing the 3'-untranslated region of a population of DNA molecules, wherein each DNA molecule in said population of DNA molecules comprises an open reading frame and a 3'-untranslated region, said method comprising: (a) providing a population of DNA molecules, each of said DNA molecules terminating in a 3' overhang upstream of the open reading frame and in a blunt end downstream of the open reading frame, wherein each of said DNA molecules is double-stranded; and (b) treating each of said DNA molecules first with an exonuclease III and then with a single-stranded nuclease under conditions that allow removal of said 3'-untranslated region, wherein the sequential treatment of said DNA molecules with the exonuclease III and the nuclease specifically removes the 3'-untranslated region from the end downstream of the open reading frame.

Specifically, the Examiner asserts that Hotz et al. teach "(a) providing a population of DNA molecules, each of said DNA molecules terminating at its 5'end in an overhang (see page 2, col. 2, paragraph 2.1, page 3, col. 1, line 1-13[)] indicates the DNA molecules amplified by using primer with restriction sites for ApaI (G/GGCCC or GGGCC/C) and XhoI (C/TCGAG) generates 5' end [over hangs] and 3' end blund end) . . ." See Office Action, page 3, lines 13-16.

Applicants respectfully submit that the Examiner has mischaracterized Hotz's method.

Hotz et al. disclose amplification of DNA molecules by using primers containing ApaI and XhoI sites, insertion of the PCR products into the pHD 260 vector, and subsequent digestion of the DNA molecules with exonuclease and mung bean nuclease (see, page 2, col. 2, paragraph 2.1; page 3, col. 1, lines 1-13). Hotz et al. include ApaI and XhoI restriction sites in the primer CZ274 (CATTGGATCCGGGCCCATGACTCGAGAGCACTGAACTTAATCATCTG, ApaI and XhoI sites underlined). Digestion with the ApaI enzyme would generate 3' overhangs due to its recognition site GGGCC/C (see a printout on ApaI from New England Biolabs, enclosed herewith as **Exhibit A**). Digestion with XhoI would generate 5' overhangs due to its recognition site C/TCGAG. Accordingly, digestion of both ApaI and XhoI enzymes could not possibly generate a blunt end at either end of the DNA molecules, contrary to the Examiner' assertion. It appears to be the Examiner's belief that the ApaI enzyme has two restriction sites (G/GGCCC or GGGCC/C) such that digestion with the ApaI enzyme will generate blund ends. However, as shown in Exhibit A, the ApaI enzyme has only one restriction site (i.e., GGGCC/C) and yields a 3' overhang. Thus, Hotz et al. at least fail to teach step (a) of claim 1.

In view of the above, Applicants respectfully submit that Hotz et al. fail to meet the limitations of the present claims and thus fail to anticipate the claimed subject matter. Reconsideration and withdrawal of this rejection are respectfully requested.

Claim Rejections under 35 U.S.C. § 102(e)

Claims 1-3 are rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Treco et al. (US Patent No. 6,063,630). See Office Action, page 4, lines 3-19.

However, Applicants note that this rejection appears to have been withdrawn. Indeed, the Examiner acknowledges that "[w]ith regard to the rejection made in the previous office action under 35 USC 102(e) as anticipated by Treco et al., Applicants' arguments filed on July 22, 2005 have been fully considered but they are persuasive. The rejection is withdrawn in view of persuasive arguments." See Office Action, page 8, lines 13-16. Clarification is respectfully requested.

Claim Rejections under 35 U.S.C. § 103(a)

Claims 4-11 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hotz et al. in view of Szostak et al. (US Patent No. 6,214,553). Applicants respectfully traverse these rejections.

As discussed above, Hotz et al. fail to anticipate the subject matter as recited in independent claim 1. Specifically, Hotz et al. fail to teach at least step (a) of claim 1 (i.e., the particular structure of the DNA molecule. Applicants submit that Szostak et al. cited by the Examiner fail to bridge the gap between Hotz et al. and the claimed invention. Further, in the absence of any direction in the art that the DNA molecules should terminate in a 3' DNA chain overhang upstream of the open reading frame and in a blunt end downstream of the open reading frame, one of ordinary skill in the art would have had no motivation to modify Hotz's method to arrive at the present invention. Thus, the Examiner has not established a *prima facie* case of obviousness in this case.

In view of the above, Applicants submit that independent claim 1 is patentably non-obvious over Hotz et al. In addition, Applicants submit that all claims depending from claim 1 recite further limitations thereon, and hence are a fortiori patentably non-obvious over Hotz et al. Reconsideration and withdrawal of rejection under 35 U.S.C. § 103(a) is respectfully requested.

Claim Rejections under 35 U.S.C. § 112, 2nd Paragraph

Claims 1 and 3-11 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Examiner asserts that the recitation "DNA molecules terminating at its 5' end in a 3' DNA chain overhang" in claim 1 is unclear and indefinite as to whether the recited "3' DNA chain overhang" refers to a complementary DNA strand of a double stranded DNA molecule at its 5' end or it refers to the single strand DNA molecule having 5' end. See Office Action, page 7, lines 15-21.

Applicants contend that one of ordinary skill in the art in the context of molecular cloning technologies would readily understand that the recitation "3' DNA chain overhang" refers to a complementary DNA strand of a double stranded DNA molecule at the 5' end of the double stranded DNA molecule. Nevertheless, solely to expedite prosecution of the application, Applicants have amended claim 1 to clarify that each of said DNA molecules terminates in a 3' overhang upstream of the open reading frame and in a blunt end downstream of the open reading frame, wherein each of said DNA molecules is double-stranded. Support for the claim amendments can be found throughout the specification (e.g., page 12, lines 7-14; and Figure 1).

Accordingly, all the pending claims as amended are clear and definite to one of skill in the art. The Examiner is respectfully requested to reconsider and withdraw all rejections under 35 U.S.C. § 112, second paragraph.

CONCLUSION

For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants believe that the claims are now in condition for allowance and early notification to this effect is earnestly solicited. Any questions arising from this submission may be directed to the undersigned at (617) 951-7000.

Applicant believes no further fee is due with this response in addition to the fees provided for on the Amendment Transmittal. However, if a further fee is due, please charge our Deposit Account No. 18-1945, under Order No. COTH-P03-504 from which the undersigned is authorized to draw.

Dated: February 6, 2006

Respectfully submitted,

By _____

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RELATED INFORMATION
- FAQs for Restriction Endonucleases
- Restriction Endonucleases Technical Reference

FAVORITE TOOLS
- Enzyme Finder
- NEBcutter
- NEBuffer Chart
- Double Digest Finder
- Isoschizomers
- DNA Sequences and Maps
- REBASE

RELATED PRODUCTS
Reagents Sold Separately
- NEBuffer 4
- BSA

Competent Cells Introductory Offer

Apa I

RR NEB4 BSA 25° Yes dcm

Catalog #	Size	Concentration	Price	Qty.	
R0114S	5,000 units	50,000 units/ml	$63.00	1	ADD TO CART
R0114L	25,000 units	50,000 units/ml	$252.00	1	ADD TO CART

Prices are in US dollars and valid only for US orders.

Download: MSDS PDF

Recognition Site:

5′...GGGCC▼C...3′
3′...C▲CCGGG...5′

isoschizomers | compatible ends | single letter code

Source:
A E. *coli* strain that carries the Apa I gene from *Acetobacter pasteurianus* sub. *pasteurianus* (ATCC 9432).

Reagents Supplied:
NEBuffer 4
BSA

Enzyme Properties

Activity in NEBuffers:
NEBuffer 1: 25%
NEBuffer 2: 50%
NEBuffer 3: 0%
NEBuffer 4: 100%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

Methylation Sensitivity:
dam methylation: Not sensitive
dcm methylation: Blocked by overlapping
CpG methylation: Blocked by overlapping

Activity at 37°C:
100%

Heat Inactivation:
65°C for 20 minutes

Survival in a Reaction:
Minimum units to digest 1 µg of substrate DNA in 16 hours: 0.13 unit(s)

Reaction & Storage Conditions

Reaction Conditions:
1X NEBuffer 4

Supplemented with 100 µg/ml Bovine Serum Albumin
Incubate at 25°C.

1X NEBuffer 4:
20 mM Tris-acetate
50 mM potassium acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition:
One unit is defined as the amount of enzyme required to digest 1 µg of Adenovirus-2 DNA in 1 hour at 25°C in a total reaction volume of 50 µl.

Concentration:
50,000 units/ml

Unit Assay Substrate:
Adenovirus-2 DNA

Storage Conditions:
10 mM Tris-HCl
50 mM KCl
1 mM dithiothreitol
0.1 mM EDTA
500 µg/ml BSA
50% glycerol
pH 7.4 @ 25°C

Storage Temperature:
-20°C

Diluent Compatibility:
Diluent A

Notes

General notes:
1. Apa I is an isoschizomer of Bsp120 I, but yields a 3' extension.

Usage notes:
1. Incubation at 37°C results in 100% activity, however, the half-life of Apa I at 37°C is only 30 minutes.
2. Apa I is inhibited by salt concentrations above 50 mM.

Quality Control

Ligation and Re-cutting:
After a 10-fold overdigestion with Apa I, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of 1-2 µM) at 16°C. Of these ligated fragments, > 95% can be recut with Apa I.

16-Hour Incubation:
A 50 µl reaction containing 1 µg of DNA and 100units of Apa I incubated for 16 hours at 25°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity:
Incubation of a 50 µl reaction containing 100 units of Apa I with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (200,000 cpm/µg) for 4 hours at 25°C released < 0.1% of the total radioactivity.

Endonuclease Activity:
Incubation of a 50 µl reaction containing 100 units of Apa I with 1 µg of ΦX174 RF I DNA for 4 hours at 25°C resulted in < 20% conversion to RFII as determined by agarose gel electrophoresis.

Blue/White Screening Assay:
An appropriate vector is digested at a unique site within the *lacZ*α gene with a 10-fold excess of

enzyme. The vector DNA is then ligated, transformed, and plated onto Xgal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, removal of even a single base gives rise to a white colony. Enzyme preparations must produce fewer than 3% white colonies to be Blue/White certified.

Quality control values for a specific lot can be found on the datacard which accompanies each product.

Reagents Sold Separately

NEBuffer 4
BSA